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REVIEW

Mechanisms of epithelial damage: are there parallels between bullous skin diseases and asthma?

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Over the last few years evidence has accumulated documenting the incidence of basement membrane zone (BMZ) autoantibodies in blistering skin disorders such as bullous pemphigus [1], cicatricial pemphigoid [2], linear IgA disease of adults (LAD) and chronic bullous disease of childhood (CBDC) [3,4]. Regarding their underlying aetiology and histopathology, these conditions appear to bear some resemblance to asthma. For example, parallels which may be drawn between bullous skin disorders and asthma include epithelial shedding and the presence of inflammatory cell infiltrate, especially eosinophils.

Bullous skin disorders are clinically characterized by the formation of subepidermal blisters or bullae which may, in a high proportion of cases, be associated with the presence of bound and circulating BMZ autoantibodies. It is clear that pemphigus and the pemphigoid diseases represent a complex array of conditions, and differentiating between the many bullous variants (termed pemphigoid as they are pemphigus-like syndromes) can present difficulties. However, conditions such as epidermolysis bullosa aquisita [5], cicatricial pemphigoid [2], and pemphigoid vegetans [6], have been distinguished. The occurrence *in vivo* of bound and circulating autoantibodies to basement membrane in these diseases prompts a number of questions. The nature of the specific antigen(s) involved, for example, have been elucidated in only a few cases [2,7,8]. Further, the trigger for autoantibody formation is unknown, or indeed whether autoantibody formation is a primary event leading to disease symptoms or a secondary event.

Autoantibodies in bullous disorders are detectable in skin biopsies at the site of lesion and in serum by direct and indirect immunofluorescence techniques [4,5]. In biopsies of bullous skin, autoantibodies are localized to the basement membrane and may additionally be localized to the intercellular region of epidermal cells [4]. Allied to the presence of autoantibodies may be the presence of linear deposits of complement factors similarly distributed along the basement membrane. Variations in the class of autoantibody and coincidence of complement factors exist between bullous disorders generally, between patients with the same type of bullous disorder, and even between different anatomical sites on the same patient [3]. BMZ autoantibodies are polyclonal in nature, with certain antigen specificities occurring more commonly than others. Some of the antigens involved in generating BMZ autoimmunity have recently been characterized, these include hemidesmosomal constituents [7] and basement membrane (BM) collagen [8].

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Basement membrane structures provide a substratum for the attachment of cellular sheets, for example epithelium, endothelium and, in the case of the skin, the epidermis. The BM is composed of collagen, glycoproteins (laminin and entactin) and specific proteoglycans [9]. Epithelial and endothelial BM are characterized by the presence of type IV collagen, which exists as heterodimers of α_1 and α_2 chains, the major BM components while the minor components, the α_3 and α_4 chains, have been reported in glomerular BM. Type IV collagen is unique from other collagens as it retains a non-collagenous domain (NC_1) due to a lack of proteolysis during processing. It is the NC_1 domain that provides the epitope for Goodpasture's antibodies. Goodpasture, in 1919 [10], described patients with glomerular nephritis and pulmonary haemorrhage which was later found to be caused by basement membrane autoimmunity [11]. Type IV collagen forms hexamers with two adjacent triple-helices sequestering the Goodpasture's epitope within the double-helix arrangement [8]. This suggests that the Goodpasture's epitope must be exposed before autoantibody generation, thus indicating a prior insult or defective collagen IV assembly.

The hemidesmosome (HD) is a complex structure binding the basal aspect of epithelial cells to the extracellular matrix substratum by intermediate sized filaments attaching into plaques on the cytoplasmic surface of the cell membrane. The HD is quite distinct from the desmosome which facilitates cell-cell adherence, bridging the intercellular space. Hemidesmosomes are

made up of five or more proteins, including integrins ($\alpha 6\beta 4$) and mediate adhesion. Nishizawa *et al.* recently characterized the major transmembrane hemidesmosomal glycoprotein as the 180 kDa (HD4) bullous pemphigoid antigen (BPAG2) [7]. In addition to this extracellular antigen, a major 230 kDa plaque protein (BP230, BPAG1) appears to be a major intracellular target for bullous pemphigus autoantibodies. It is known that the $\alpha 6$-$\beta 4$ heterodimer associates with the BPAG2 and the anchorage filaments composed of type VII collagen [12]. The incidence of the $\alpha 6$-$\beta 4$ integrin complex presents one criterion for distinguishing BP from other variants such as epidermolysis bullous aquisita, bullous systemic lupus erythematosus, and pemphigus vulgaris as the integrin complex in bullous pemphigus is absent or patchy in distribution while in the other conditions it is normally distributed [13]. Autoantibodies generated against basal cell substratum and HD thus undermine the integrity of the epithelium by associating with the BM type IV collagen (Goodpasture's epitope) and targeting the 180 kDa and 230 kDa HD antigens.

In asthma, areas of epithelium become denuded of columnar cells leaving the exposed basal cells firmly attached to the basement membrane. Desmosomes are distributed along the basal and lateral aspect of columnar cells of the normal bronchial epithelium, but not between the basal cells and the BM [14]. Disruption of the desmosomes could therefore account for the pattern of bronchial epithelial loss associated with asthma. In this case, the loss of epithelial integrity has been suggested to be due to fragility of the desmosomes mediated via eosinophil derived proteins [14]. The involvement of autoantibodies directed at desmosomal targets in asthma is unknown and remains to be investigated.

The presence of BMZ autoantibodies in blistering disorders may be responsible for disease symptoms, however, this need not necessarily be the case. *Herpes Gestationis* (HG) is characterized by a high incidence (30–40%) of epidermal BM autoantibodies of the IgG class [15]. Stone [15] proposed that the appearance of autoantibody is a secondary event in HG, a condition associated with the last 4 months of pregnancy (and postpartum) and producing bullous type symptoms. Increased oestrogen and progesterone levels were postulated to increase tissue viscosity, hindering the dispersal of tissue fluid and leading to blister formation. The presence of BM autoantibodies was proposed to be secondary to the cell and tissue trauma associated with the formation of bullous lesions. Similarly, it has been proposed that epithelial fragility in asthma may occur through cleavage of desmosomes secondary to epithelial oedema [16]. In HG, as in many bullous syndromes, there appears to be an association with an inflammatory cell infiltrate predominantly made up of eosinophils, which may be the cause of BM disruption [5,15,17]. The release of major basic protein (MBP) from eosinophils and its subsequent association with antigens present in damaged tissue was proposed [15] to present the immune system with a novel antigenic complex, leading to autoantibody synthesis and possibly providing an inflammatory cell chemotactic stimulus. Interestingly, it has been shown that a constituent of blister fluid converted normodense guinea-pig eosinophils to a hypodense, activated state [18]. This was reflected in increased helminthotoxic capacity, enhanced chemiluminescence response to opsonized zymosan and augmented expression of cell surface receptors in eosinophils treated with blister fluid. This, however, provides no explanation for the accumulation of eosinophils prior to autoantibody formation.

The eosinophil plays a central role in the pathogenesis of asthma, especially in bronchial epithelial shedding [14]. The bronchial epithelium of the asthmatic individual seems to be inherently more fragile than normal subjects and, therefore, predisposed to shedding. The consequences of epithelial loss include increased bronchial hyperresponsiveness due to exposed intra-epithelial peptidergic nerves, reduction of epithelial-derived relaxant factor and production of proinflammatory mediators. The eosinophil has been suggested to be responsible for the loss of epithelial integrity at the basal cell-columnar cell junction due to desmosomal disruption [16]. The growing evidence for eosinophil involvement in bronchial epithelial damage indicates that loss of superficial cells may occur through eosinophil mediated release of metalloproteinases from epithelial cells [16].

The presence of activated complement at sites of autoantibody binding may provide a further mechanism for epithelial damage whereby autoantibody-antigen associations provide the stimulus for the activation of complement. In cases of ulcerative colitis expression of the M_r 40 kDa antigen, to which IgG1 autoantibodies are produced, co-localized with C3b at the apices of colonic enterocytes [19]. This was despite the fact that antigen expression was somewhat reduced in regions of colonic epithelium where the action of complement had been extensive. Halstenen *et al.* [19] proposed, therefore, that an autoimmune response to the 40 kDa antigen, leading to complement activation mediated by IgG1, was the pathogenetic mechanism for epithelial damage and persistent inflammation in ulcerative colitis. Also, in cicatricial pemphigoid, IgA is associated with deposits of properdin and properdin factor B indicating IgA activates the alternative complement pathway [2]. The

association of autoantibodies and complement does not always occur. In a case of pemphigous vegitans IgG4 (a comparatively ineffective complement activator) was localized to the BMZ in the absence of an C3 deposits [6]. It is noted that in this case of pemphigous vegetans a diffuse eosinophilia was present.

The precise aetiology of BMZ autoantibody generation is still unclear, however, some interesting observations have been reported. It has been shown, for example, that IFN-α treatment of carcinoid tumours produces autoantibodies to epithelia and human carcinoid tumour cells [20]. As yet the mechanism for autoantibody generation in IFN-α treated patients is unclear, however, the aberrant expression of altered cytoskeletal elements such as the cytokeratins and MHC II antigens could provide the necessary stimulus to evoke an immune response. *In vitro* experiments have demonstrated the production of anti-BMZ autoantibodies in Epstein-Barr virus transformed lymphocytes from BP patients [21]. These lymphocytes produced three monoclonal antibodies, two of which reacted with the 230 kDa BP antigen. A mouse model of retinopathy initiated by infection with murine coronavirus resulted in the production of autoantibodies to retinal pigmented epithelial cells and the glial cell component, the Müller cell [22]. The production of autoantibodies to retinal pigmented epithelial cells may be significant as these cells are believed to play a role in antigen presentation. Thus, the presence of the viral antigen within this cell at an early time in the disease process, may contribute to the autoimmune reactions observed.

The role of respiratory viruses in airway allergic conditions, including asthma, is thought to be important in genetically predisposed individuals [23]. Precipitation of asthmatic symptoms by different viruses appears to be dependent on age. During childhood respiratory syncitial virus is the most potent stimulator of wheezing symptoms, while rhinovirus, influenza and parainfluenza viruses produce similar effects as age increases. Viruses influence bronchial smooth muscle function by a variety of means including sensitization with IgE, epithelial shedding, eosinophil recruitment, and promotion of mediator release from leucocytes. Airway hyper-responsiveness in viral infected groups may involve alteration in both autonomic (increased vagal sensitivity and reduced β-adrenoceptor responses) and neuropeptidergic control. Additionally, even though the precise role of the basophil is unclear a positive correlation between the degree of histamine release (presumably from mast cells and basophils bearing virus-specific IgE) and the magnitude of airway reactivity in asthma has been observed [23].

Not all blistering skin disorders are due to autoimmunity. Epidermolysis bullosa simplex (EBS), for example, is an autosomal recessive disorder. Coulombe *et al.* (1991) showed this rare condition (1 : 50 000) was due to specific cytokeratin 5 and 14 polymorphisms leading to an amino acid substitution (arg to cys) at the highly conserved 125 position [24]. This particular mutation disrupts keratin network formation leading to basal cell cytolysis and the onset of blistering [25]. This illustrates the diverse events that may result in blistering skin. The example of keratin polymorphisms, as yet, has no parallel in asthma. Also, it is possible to exhibit BMZ autoantibodies without the usual symptoms. For example, a case of an individual with IgG and C3 deposits along the alveolar basement membrane and IgM and C3 along the glomerular basement membrane exhibited pulmonary haemorrhage but with no disruption to renal function [26]. Conversely, the depletion of BMZ autoantibodies is not a pre-requisite for remission of bullous conditions, although in one study 71% of patients with LAD or CBDC lost the characteristic BMZ autoreactivity during remission [3]. The difficulty in establishing any one factor in autoimmune syndromes is illustrated by the data on Behcet's disease which is a major vascular occluding disorder mediated by autoendothelial cell autoantibodies [27]. Generally, but not in all cases, Behcet's disease was found to correlate with specific autoantibody, and no single antibody isotype correlated with any of the different clinical signs typical of this disease. This condition is known to be complicated by factors such as genetic predisposition, viral and bacterial infection as well as environmental factors [27]. Additionally, endothelial cell lysis by autoantibodies may be dependent on prior exposure of endothelial cells to the pro-inflammatory cytokines IL-1 and tumour necrosis factor-(TNF)-α [28].

The fundamental issue as to why autoantibodies are generated can be addressed from at least two possible perspectives. The first considers this phenomenon as a faulty or inappropriate immune response, to infection for example. Hooks *et al.* (1993) discussed a number of possible reasons for post-infection autoantibody synthesis including the introduction of foreign material into the host that resembles native molecules, possible changes in the hosts own antigen or disruption of the immune system [22]. The second considers the normal production of autoantibodies to enhance the debridement of damaged (injured or infected) tissues [15], indicating that the inappropriate expression of a normal biological function may lead to the lesions described above. Were it to be shown that the incidence of autoantibodies in conditions such as BP was central to the aetiology of the disease, treatment may hinge on systemic removal of the autoantibodies. Such an

approach was successfully utilized by Bernard *et al.* (1993) for a patient with a potentially fatal case of autoimmune neutropenia and thrombocytopenia [29]. Removal of circulating autoantibodies to neutrophils (IgG) and platelets (IgG and IgM) was achieved by immunoadsorption onto a protein-A-column, and combined with chemotherapy and oral steroid administration. Whether such a technique would be economically feasible for BP conditions is outside the scope of this article. Nevertheless, such conditions are rare and may warrant specialized treatment when conventional therapies prove ineffective.

As yet there is no evidence to suggest asthmatic subjects are predisposed to bullous disorders or vice versa. It is possible, however, that similar stimuli precipitate both conditions in susceptible individuals. For now this area of research is generating more questions than answers, which will be essential for our further understanding of the pathogenesis of these conditions.

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